L5 ANSWER 2 OF 5 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2001316545 MEDLINE
DOCUMENT NUMBER: 21283080 PubMed ID: 11389184
TITLE: Functional expression of M(1), M(3) and M(5) muscarinic acetylcholine receptors in **yeast**.
AUTHOR: Erlenbach I; Kostenis E; Schmidt C; Hamdan F F; Pausch M H; Wess J
CORPORATE SOURCE: Laboratory of Bioorganic Chemistry, NIDDK, National Institutes of Health, Bethesda, MD 20892, USA.
SOURCE: JOURNAL OF NEUROCHEMISTRY, (2001 Jun) 77 (5) 1327-37. Journal code: 2985190R. ISSN: 0022-3042.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200107
ENTRY DATE: Entered STN: 20010716
Last Updated on STN: 20010716
Entered Medline: 20010712

AB The goal of this study was to functionally express the three G(q)-coupled muscarinic receptor subtypes, M(1), M(3) and M(5), in **yeast** (Saccharomyces cerevisiae). Transformation of **yeast** with expression constructs coding for the full-length receptors resulted in very low numbers of detectable muscarinic binding sites (B(max) < 5 fmol/mg). Strikingly, **deletion** of the central portion of the third **intracellular loops** of the M(1), M(3) and M(5) muscarinic receptors resulted in dramatic increases in B(max) values (53-214 fmol/mg). To monitor productive receptor/**G-protein** coupling, we used specifically engineered **yeast** strains that required agonist-stimulated receptor/**G-protein** coupling for cell growth. These studies showed that the shortened versions of the M(1), M(3) and M(5) receptors were unable to productively interact with the endogenous **yeast G protein** alpha-subunit, Gpa1p, or a Gpa1 mutant subunit that contained C-terminal mammalian Galpha(s) sequence. In contrast, all three receptors gained the ability to efficiently couple to a Gpa1/Galpha(q) hybrid subunit containing C-terminal mammalian Galpha(q) sequence, indicating that the M(1), M(3) and M(5) muscarinic receptors retained proper **G-protein** coupling selectivity in **yeast**. This is the first study to report the expression of muscarinic receptors in a coupling-competent form in **yeast**. The strategy described here, which involves structural modification of both receptors and co-expressed G proteins, should facilitate the functional expression of other classes of **G protein**-coupled receptors in **yeast**.

L5 ANSWER 1 OF 5 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2001453364 MEDLINE
DOCUMENT NUMBER: 21369918 PubMed ID: 11375990
TITLE: Single amino acid substitutions and **deletions** that alter the **G protein** coupling properties of the V2 vasopressin receptor identified in **yeast** by receptor random mutagenesis.
AUTHOR: Erlenbach I; Kostenis E; Schmidt C; Serradeil-Le Gal C; Raufaste D; Dumont M E; Pausch M H; Wess J
CORPORATE SOURCE: Laboratory of Bioorganic Chemistry, NIDDK, National Institutes of Health, Bethesda, Maryland 20892, USA.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Aug 3) 276 (31) 29382-92.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200109
ENTRY DATE: Entered STN: 20010814
Last Updated on STN: 20030105
Entered Medline: 20010913

AB To facilitate structure-function relationship studies of the V2 vasopressin receptor, a prototypical G(s)-coupled receptor, we generated V2 receptor-expressing **yeast** strains (Saccharomyces cerevisiae) that required arginine vasopressin-dependent receptor/**G protein** coupling for cell growth. V2 receptors heterologously expressed in **yeast** were unable to productively interact with the endogenous **yeast G protein** alpha subunit, Gpa1p, or a mutant Gpa1p subunit containing the C-terminal G alpha(q) sequence (Gq5). In contrast, the V2 receptor efficiently coupled to a Gpa1p/G alpha(s) hybrid subunit containing the C-terminal G alpha(s) sequence (Gs5), indicating that the V2 receptor retained proper **G protein** coupling selectivity in **yeast**. To gain insight into the molecular basis underlying the selectivity of V2 receptor/**G protein** interactions, we used receptor saturation random mutagenesis to generate a **yeast** library expressing mutant V2 receptors containing mutations within the second **intracellular loop**. A subsequent **yeast** genetic screen of about 30,000 mutant receptors yielded four mutant receptors that, in contrast to the wild-type receptor, showed substantial coupling to Gq5. Functional analysis of these mutant receptors, followed by more detailed site-directed mutagenesis studies, indicated that single amino acid substitutions at position Met(145) in the central portion of the second **intracellular loop** of the V2 receptor had pronounced effects on receptor/**G protein** coupling selectivity. We also observed that **deletion** of single amino acids N-terminal of Met(145) led to misfolded receptor proteins, whereas single amino acid **deletions** C-terminal of Met(145) had no effect on V2 receptor function. These findings highlight the usefulness of combining receptor random mutagenesis and **yeast** expression technology to study mechanisms governing receptor/**G protein** coupling selectivity and receptor folding.

L5 ANSWER 5 OF 5 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 94179290 MEDLINE
DOCUMENT NUMBER: 94179290 PubMed ID: 8132618
TITLE: Systematic mutagenesis of the **yeast** mating pheromone receptor third **intracellular loop**.
AUTHOR: Clark C D; Palzkill T; Botstein D
CORPORATE SOURCE: Department of Genetics, Stanford University School of Medicine, California 94305.
CONTRACT NUMBER: GM46406 (NIGMS)
GM46888 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1994 Mar 25) 269 (12) 8831-41.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199404
ENTRY DATE: Entered STN: 19940428
Last Updated on STN: 20000303
Entered Medline: 19940421

AB Signal transduction in the mating pathway of the **yeast** Saccharomyces cerevisiae is initiated by binding of a peptide pheromone to a **G protein**-coupled receptor (Ste2). We systematically have mutated the third **intracellular loop** of the Ste2 receptor to investigate its functional significance. We substituted each of the 13 amino acids in the loop with alanine individually or together with one other residue. In addition, we used a site-directed random replacement mutagenesis technique to replace a region encoding three amino acids in the loop with random sequence. Over 80 such Ste2 mutants have been analyzed by several functional and biochemical criteria in a **yeast** strain that carries a genomic **deletion** of the STE2 gene. The mutant phenotypes range from fully functional to severely compromised in signaling. The observation that amino acid substitutions in the third **intracellular loop** of the Ste2 receptor can affect activation of the **yeast** mating response implicates the loop in this signal transduction pathway. The types of mutations that compromise the function of the receptor may provide clues to the physical interaction between the receptor and the **G protein**.

L7 ANSWER 20 OF 21 MEDLINE DUPLICATE 16
ACCESSION NUMBER: 92317060 MEDLINE
DOCUMENT NUMBER: 92317060 PubMed ID: 1618842
TITLE: Hm1 muscarinic cholinergic receptor **internalization** requires a domain in the third cytoplasmic loop.
AUTHOR: Lameh J; Philip M; Sharma Y K; Moro O; Ramachandran J; Sadee W
CORPORATE SOURCE: Department of Pharmacy, University of California, San Francisco 94143.
CONTRACT NUMBER: DA 04166 (NIDA)
GM 43102 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1992 Jul 5) 267 (19) 13406-12.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199208
ENTRY DATE: Entered STN: 19920815
Last Updated on STN: 19980206
Entered Medline: 19920805

AB Selected regions of the Hm1 muscarinic cholinergic receptor were mutated to analyze the molecular mechanisms of agonist-induced receptor **internalization** (or sequestration). The wild-type and mutant Hm1 genes were expressed, using pSG5, in U293 human kidney cells. Whereas surface receptor density measured with the polar tracer N-[3H]methylscopolamine was rapidly reduced by carbachol exposure, total receptor content measured with [3H]quinuclidinyl benzilate did not decline for at least 24 h, indicating the absence of extensive receptor down-regulation in U293 cells. Carbachol stimulation of phosphatidylinositol turnover paralleled receptor **internalization**, both with EC50 values of 10-20 microM. Furthermore, a D71N point mutation that prevented receptor activation also abolished carbachol-induced receptor **internalization**, indicating that receptor activation (but not necessarily second messenger stimulation) was required for **internalization**. Truncation of the COOH-terminal tail (K447 trunc) and point mutations of several potential Ser and Thr phosphorylation sites to Ala failed to affect receptor activation and **internalization**. In contrast, partial **deletions** of the third **intracellular loop** (i3) (Tyr208-Thr366) resulted in receptor mutants deficient in agonist-induced receptor **internalization**/sequestration. Various **deletions** caused either complete loss of **internalization** (d 232-358) or impaired **internalization**, ranging from 10 to 30% over 2 h, whereas wild-type Hm1 internalized to approximately 50%. Whereas the reason for the observed differences among the deficient **deletion** mutants remains unclear, the initial rate of N-[3H]methylscopolamine binding loss from the cell surface was much slower than that of wild-type Hm1 in each case. The **deletion** of only one single domain, 284-292 (SMESLTSSE), in the middle of i3 was consistently associated with impaired **internalization**. Domain 284-292 is partially conserved among closely related muscarinic receptors, whereas most of the remainder of i3 is not (except for the i3 membrane junctions), and similar Ser- and Thr-rich regions are present in many other **G protein**-coupled receptors. We propose that a small receptor domain in the middle of the i3 loop of Hm1 is involved in agonist-induced receptor **internalization**.

L7 ANSWER 19 OF 21 MEDLINE DUPLICATE 15
ACCESSION NUMBER: 94067159 MEDLINE
DOCUMENT NUMBER: 94067159 PubMed ID: 8247012
TITLE: Decreased levels of internalized thyrotropin-releasing hormone receptors after uncoupling from guanine nucleotide-binding protein and phospholipase-C.
AUTHOR: Nussenzveig D R; Heinflink M; Gershengorn M C
CORPORATE SOURCE: Department of Medicine, Cornell University Medical College, New York Hospital, New York 10021.
CONTRACT NUMBER: DK-43046 (NIDDK)
SOURCE: MOLECULAR ENDOCRINOLOGY, (1993 Sep) 7 (9) 1105-11. Journal code: 8801431. ISSN: 0888-8809.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199401
ENTRY DATE: Entered STN: 19940201
Last Updated on STN: 20000303
Entered Medline: 19940104

AB **Internalization** of TRH receptor (TRH-R) is dependent on sequences/structures in the receptor carboxyl-terminal tail. Here, we studied whether coupling to guanine nucleotide-binding protein (**G -protein**) and phospholipase-C (PLC) is involved in **internalization**. We constructed two mutant TRH-Rs: delta 218-263 TRH-R, in which most of the residues that form the putative third **intracellular loop** were **deleted**, and D71A TRH-R, in which an Asp in the putative second transmembrane helix was mutated to Ala; these TRH-Rs did not activate PLC when expressed transiently in COS-1 cells. In contrast to wild-type (WT) TRH-Rs, approximately 60% of which were internalized at steady state after binding methyl-HisTRH, only approximately 15% of delta 218-263 and D71A TRH-Rs were internalized. Thus, mutant TRH-Rs that do not activate PLC, most likely because they are uncoupled from G-proteins, are internalized to lesser extents than WT TRH-Rs. We also studied the effects of U73122 (1-[6-[[17 beta-3-methoxyestra-1,3,5(10)-trien-17-yl]amino] hexyl]-1H-pyrrole-2,5-dione), an amino steroid that inhibits receptor-mediated activation of PLC. In COS-1 and AtT-20 cells transfected with WT TRH-Rs and in GH3 cells, U73122 virtually abolished TRH activation of PLC and partially reduced the fraction of WT TRH-Rs internalized. Thus, uncoupling WT TRH-Rs from PLC decreases **internalization**. We conclude that TRH-R coupling to **G-protein** and PLC increases the number of TRH-Rs internalized at steady state even though the primary signals for agonist-induced **internalization** are present in the receptor. These data support the idea that a quaternary complex of TRH/TRH-R/**G protein**/PLC is normally internalized.

L7 ANSWER 18 OF 21 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 93216613 MEDLINE
DOCUMENT NUMBER: 93216613 PubMed ID: 8463213
TITLE: Serine- and threonine-rich domain regulates **internalization** of muscarinic cholinergic receptors.
AUTHOR: Moro O; Lameh J; Sadee W
CORPORATE SOURCE: Department of Pharmacy, University of California, San Francisco 94143-0446.
CONTRACT NUMBER: GM 43102 (NIGMS)
K21 MH 00996 (NIMH)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Apr 5) 268 (10) 6862-5.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199305
ENTRY DATE: Entered STN: 19930521
Last Updated on STN: 19930521
Entered Medline: 19930505

AB Upon agonist exposure, most membrane receptors internalize into the cell as part of an adaptation process. Receptor domains that mediate **internalization** have been defined for several receptor classes, e.g. growth factor and transport receptors, but not yet for any of the numerous members of the family of **G protein**-coupled receptors (GPCRs), having seven putative transmembrane helices. With the use of **deletion** mutations, we previously showed that a small region in the middle of the third **intracellular loop** (i3) appears to be required for the agonist-induced **internalization** of the Hm1 muscarinic cholinergic receptor (Lameh, J., Philip, M., Sharma, Y. K., Moro, O., Ramachandran, J., and Sadee, W. (1992) J. Biol. Chem. 267, 13406-13412). Using point mutations, we now demonstrate that domain 286-292 (ESLTSSE) is required, with TSS playing a crucial role. Although the i3 loops of GPCRs share minimal overall sequence identity, even among closely related subtypes, small domains containing multiple S/T residues are also present in several other GPCRs, e.g. peptide, catechol, and all the muscarinic cholinergic receptor subtypes. S/T-->A mutations in analogous i3 domains also prevented receptor **internalization** in the case of Hm3 and attenuated **internalization** of Hm2. We propose that an S/T-rich domain in the i3 loop, possibly via phosphorylation reactions, regulates one pathway of **GPCR internalization**.

L7 ANSWER 17 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1994:387539 BIOSIS
DOCUMENT NUMBER: PREV199497400539
TITLE: **Deletion** analysis of the m4 muscarinic acetylcholine receptor: Molecular determinants for activation of but not coupling to the G-i guanine-nucleotide-binding regulatory protein regulate receptor **internalization**.
AUTHOR(S): Van Koppen, Chris J. (1); Sell, Alexandra; Lenz, Wolfgang; Jakobs, Karl H.
CORPORATE SOURCE: (1) Inst. Pharmakol., Univ. GH Essen, Hufelandstrasse 5, D-45122 Essen Germany
SOURCE: European Journal of Biochemistry, (1994) Vol. 222, No. 2, pp. 525-531.
ISSN: 0014-2956.
DOCUMENT TYPE: Article
LANGUAGE: English
AB In order to investigate whether coupling to and/or activation of guanine-nucleotide-binding proteins (G proteins) is involved in agonist-induced **internalization** of m4 muscarinic acetylcholine receptors (mAChRs), a **deletion** mutant (des-(264-394)mAChR) was constructed that lacks a substantial portion of the putative third **intracellular loop**. The wild-type receptor and des-(264394)mAChR stably expressed in Chinese hamster ovary cells in essentially comparable amounts, exhibited identical antagonist-binding affinities. Consistent with the reported importance of the third cytoplasmic loop for G-i protein activation, the des-(264-394)mAChR showed a drastically reduced potential to mediate agonist-induced inhibition of adenylyl cyclase. In contrast, the ability of the mutant receptor to couple to G-i proteins was not impaired, as demonstrated by a similar guanine-nucleotide-sensitive and pertussis-toxin-sensitive high-affinity agonist-receptor binding for both mAChRs. In contrast, des-(264-394)mAChR was hardly able to stimulate the GTPase activity of G proteins, suggesting impaired activation of G-i proteins rather than ineffective coupling to G-i proteins. **Internalization** of wild-type receptor and des-(264-394)mAChR was observed with similar agonist concentrations and showed similar maximal values. However, des-(264-394)mAChR displayed a significantly reduced rate of receptor **internalization**. A similar attenuation of wild-type mAChR **internalization** was obtained upon pertussis toxin treatment. In conclusion, our data provide evidence that the molecular determinants of the m4 mAChR involved in G-i-protein coupling and activation are not identical and that activation of, but not coupling to, G-i proteins regulates m4 mAChR **internalization**.

L7 ANSWER 16 OF 21 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 95181465 MEDLINE
DOCUMENT NUMBER: 95181465 PubMed ID: 7876239
TITLE: Four consecutive serines in the third **intracellular loop** are the sites for beta-adrenergic receptor kinase-mediated phosphorylation and **desensitization** of the alpha 2A-adrenergic receptor.
AUTHOR: Eason M G; Moreira S P; Liggett S B
CORPORATE SOURCE: Department of Pulmonary Medicine, University of Cincinnati College of Medicine, Ohio 45267-0564.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Mar 3) 270 (9) 4681-8.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199504
ENTRY DATE: Entered STN: 19950419
Last Updated on STN: 19950419
Entered Medline: 19950405

AB During short term agonist exposure, the alpha 2A-adrenergic receptor (alpha 2AAR) undergoes rapid functional **desensitization** caused by phosphorylation of the receptor by the beta-adrenergic receptor kinase (beta ARK). This signal quenching is similar in nature to that found with a number of **G-protein** coupled receptors in which agonist-promoted **desensitization** is due to beta ARK phosphorylation; like these other receptors, the precise molecular determinants of the receptor required for beta ARK phosphorylation are not known. To delineate such a motif in the human alpha 2AAR (alpha 2C10), we constructed six mutated receptors consisting of **deletions** or substitutions of Ser-296-299 in the EESSSS sequence of the third **intracellular loop** of the receptor. These were expressed in Chinese hamster ovary and COS-7 cells, and agonist-promoted **desensitization** and receptor phosphorylation were assessed. **Deletion** of the EESSSS sequence and substitution of alanine for all four serines resulted in a total loss of phosphorylation and **desensitization**. Mutant receptors that retained two of the original serines (AASS and SSAA) underwent agonist-promoted phosphorylation of 55 +/- 7% and 57 +/- 8% of the phosphorylation found for wild type alpha 2C10. Additional substitution mutants (SSSA and SAAA) underwent 77 +/- 1% and 27 +/- 4% of wild type phosphorylation, respectively. Thus, substitution of alanine for each additional serine decreased overall phosphorylation as compared with wild type alpha 2C10 by approximately 25%, which is consistent with all 4 serines being phosphorylated. Mutated receptors that only partially phosphorylated (as compared with wild type) failed to undergo agonist-promoted **desensitization**. Thus, beta ARK-mediated phosphorylation of alpha 2C10 occurs at Ser-296-299 in the third **intracellular loop**, and this represents the critical step in rapid agonist-promoted **desensitization**. A number of other **G-protein** coupled receptors that undergo **desensitization** have a sequence motif similar to that which we have found for beta ARK-mediated phosphorylation of alpha 2C10, suggesting that these receptors may also be substrates for beta ARK.

L7 ANSWER 15 OF 21 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 96018886 MEDLINE
DOCUMENT NUMBER: 96018886 PubMed ID: 7547931
TITLE: An acidic motif within the third **intracellular loop** of the alpha2C2 adrenergic receptor is required for agonist-promoted phosphorylation and **desensitization**.
AUTHOR: Jewell-Motz E A; Liggett S B
CORPORATE SOURCE: Department of Medicine (Pulmonary), University of Cincinnati College of Medicine, Ohio 45267-0564, USA.
CONTRACT NUMBER: HL07382 (NHLBI)
HL41496 (NHLBI)
HL53436 (NHLBI)
SOURCE: BIOCHEMISTRY, (1995 Sep 19) 34 (37) 11946-53.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199510
ENTRY DATE: Entered STN: 19951227
Last Updated on STN: 19970203
Entered Medline: 19951030

AB The alpha 2C2 adrenergic receptor contains a highly acidic stretch of amino acids (EDEAEEEEEEEEEEE) within the third **intracellular loop**. To investigate the role of this region, we utilized site-directed mutagenesis to **delete** these 16 amino acids as well as to substitute them with glutamine, thereby conserving size but not charge. The wild-type and mutated alpha 2C2 receptors were permanently expressed in CHO cells. Neither substitution nor **deletion** of this region affected receptor expression, agonist or antagonist binding affinities, guanine nucleotide-sensitive formation of the high-affinity agonist-receptor-**G protein** complex, or functional coupling of the receptor to Gi. We considered that since alpha 2C2 agonist-promoted **desensitization** is due to phosphorylation by the beta-adrenergic receptor kinase (or a related kinase), that this region may be important for establishing the acidic mileau required by this kinase. Therefore, the consequences of 30 min of agonist preexposure on subsequent alpha 2C2-mediated inhibition of adenylyl cyclase and on high-affinity agonist binding were determined for the wild-type and these two mutants. The wild-type alpha 2C2 receptor underwent approximately 52% functional **desensitization** and a approximately 40% loss of high-affinity binding after such exposure. In contrast, **deletion** and substitution of this acidic stretch of amino acids ablated **desensitization** as assessed by both approaches. These results correlated with those obtained in whole cell phosphorylation experiments. Cells expressing each receptor were incubated with [32P]orthophosphate and exposed to agonist, and receptors were purified by immunoprecipitation. The **deletion** and the substitution mutant receptors underwent agonist-promoted phosphorylation at levels of only 44 +/- 5% and 50 +/- 15%, respectively, relative to wild-type alpha 2C2.(ABSTRACT TRUNCATED AT 250 WORDS)

L7 ANSWER 14 OF 21 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 96081975 MEDLINE
DOCUMENT NUMBER: 96081975 PubMed ID: 7499433
TITLE: **Desensitization** and **internalization** of the m2 muscarinic acetylcholine receptor are directed by independent mechanisms.
AUTHOR: Pals-Rylaarsdam R; Xu Y; Witt-Enderby P; Benovic J L; Hosey M M
CORPORATE SOURCE: Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, Illinois 60611, USA.
CONTRACT NUMBER: ES00210 (NIEHS)
GM 44944 (NIGMS)
HL 50201 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Dec 1) 270 (48) 29004-11.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199601
ENTRY DATE: Entered STN: 19960217
Last Updated on STN: 19960217
Entered Medline: 19960118

AB The phenomenon of acute **desensitization** of **G-protein**-coupled receptors has been associated with several events, including receptor phosphorylation, loss of high affinity agonist binding, receptor:**G-protein** uncoupling, and receptor **internalization**. However, the biochemical events underlying these processes are not fully understood, and their contributions to the loss of signaling remain correlative. In addition, the nature of the kinases and the receptor domains which are involved in modulation of activity have only begun to be investigated. In order to directly measure the role of **G-protein**-coupled receptor kinases (GRKs) in the **desensitization** of the m2 muscarinic acetylcholine receptor (m2 mAChR), a dominant-negative allele of GRK2 was used to inhibit receptor phosphorylation by endogenous GRK activity in a human embryonic kidney cell line. The dominant-negative GRK2K220R reduced agonist-dependent phosphorylation of the m2 mAChR by approximately 50% and prevented acute **desensitization** of the receptor as measured by the ability of the m2 mAChR to attenuate adenylyl cyclase activity. In contrast, the agonist-induced **internalization** of the m2 mAChR was unaffected by the GRK2K220R construct. Further evidence linking receptor phosphorylation to acute receptor **desensitization** was obtained when two **deletions** of the third **intracellular loop** were made which created m2 mAChRs that did not become phosphorylated in an agonist-dependent manner and did not **desensitize**. However, the mutant mAChRs retained the ability to internalize. These data provide the first direct evidence that GRK-mediated receptor phosphorylation is necessary for m2 mAChR **desensitization**; the likely sites of in vivo phosphorylation are in the central portion of the third **intracellular loop** (amino acids 282-323). These results also indicate that **internalization** of the m2 receptor is not a key event in **desensitization** and is mediated by mechanisms distinct from GRK phosphorylation of the receptor.

L7 ANSWER 13 OF 21 MEDLINE
ACCESSION NUMBER: 96413593 MEDLINE
DOCUMENT NUMBER: 96413593 PubMed ID: 8816747
TITLE: Dependence of agonist activation on a conserved apolar residue in the third **intracellular loop** of the AT1 angiotensin receptor.
AUTHOR: Hunyady L; Zhang M; Jagadeesh G; Bor M; Balla T; Catt K J
CORPORATE SOURCE: Endocrinology and Reproduction Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1996 Sep 17) 93 (19) 10040-5. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199611
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 19961219
Entered Medline: 19961113

AB The coupling of agonist-activated **seven transmembrane** domain receptors to G proteins is known to involve the amino-terminal region of their third cytoplasmic loop. Analysis of the amino acids in this region of the rat type in angiotensin (AT1a) receptor identified Leu-222 as an essential residue in receptor activation by the physiological agonist, angiotensin II (Ang II). Nonpolar replacements for Leu-222 yielded functionally intact AT1 receptors, while polar or charged residues caused progressive impairment of Ang II-induced inositol phosphate generation. The decrease in agonist-induced signal generation was associated with a parallel reduction of receptor **internalization**, and was most pronounced for the Lys-222 mutant receptor. Although this mutant showed normal binding of the peptide antagonist, [Sar1,Ile6]Ang II, its affinity for Ang II was markedly reduced, consistent with its inability to adopt the high-affinity conformation. A search revealed that many Gq-coupled receptors contain an apolar amino acid (frequently leucine) in the position corresponding to Leu-222 of the AT1 receptor. These findings suggest that such a conserved apolar residue in the third **intracellular loop** is a crucial element in the agonist-induced activation of the AT1 and possibly many other **G protein**-coupled receptors.

L7 ANSWER 12 OF 21 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 1999060049 MEDLINE
DOCUMENT NUMBER: 99060049 PubMed ID: 9843377
TITLE: Agonist-mediated downregulation of G alpha i via the alpha 2-adrenergic receptor is targeted by receptor-Gi interaction and is independent of receptor signaling and regulation.
AUTHOR: Jewell-Motz E A; Donnelly E T; Eason M G; Liggett S B
CORPORATE SOURCE: Department of Medicine, University of Cincinnati College of Medicine, Ohio 45267-0564, USA.
CONTRACT NUMBER: HL41496 (NHLBI)
HL53436 (NHLBI)
SOURCE: BIOCHEMISTRY, (1998 Nov 10) 37 (45) 15720-5.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 20000303
Entered Medline: 19981221

AB One mechanism of long-term agonist-promoted **desensitization** of alpha2AR function is downregulation of the cellular levels of the alpha subunit of the inhibitory **G protein**, Gi. In transfected CHO cells expressing the human alpha2AAR, a 40.1 +/- 3.3% downregulation of Galphai2 protein occurred after 24 h of exposure of the cells to epinephrine, which was not accompanied by a decrease in Galphai2 mRNA. The essential step that targets Gi for degradation by agonist occupancy of the receptor was explored using mutated alpha2AAR lacking specific structural or functional elements. These consisted of 5HT1A receptor and beta2AR sequences substituted at residues 113-149 of the second **intracellular loop** and 218-235 and 355-371 of the N- and C-terminal regions of the third **intracellular loop** (altered Gi and Gs coupling), **deletion** of Ser296-299 (absent GRK phosphorylation), and substitution of Cys442 (absent palmitoylation and receptor downregulation). Of these mutants, only those with diminished Gi coupling displayed a loss of agonist-promoted Gi downregulation, thus excluding Gs coupling and receptor downregulation, palmitoylation, and phosphorylation as necessary events. Furthermore, coupling-impaired receptors consisting of mutations in the second or third loops ablated Gi downregulation, suggesting that a discreet structural motif of the receptor is unlikely to represent a key element in the process. While pertussis toxin ablated Gi downregulation, blocking downstream intracellular consequences of alpha2AAR activation or mimicking these pathways by heterologous means failed to implicate cAMP/adenylyl cyclase, phospholipase C, phospholipase D, or MAP kinase pathways in alpha2AAR-mediated Gi downregulation. Taken together, agonist-promoted Gi downregulation requires physical alpha2AAR-Gi interaction which targets Gi for degradation in a manner that is independent of alpha2AAR trafficking, regulation, or second messengers.

L7 ANSWER 5 OF 21 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 2001495996 MEDLINE
DOCUMENT NUMBER: 21429708 PubMed ID: 11543875
TITLE: Identification and functional characterization of alpha(2)-adrenoceptor polymorphisms.
AUTHOR: Small K M; Liggett S B
CORPORATE SOURCE: Depts. of Medicine and Pharmacology, University of Cincinnati Colloge of Medicine, Cincinnati, OH 45327-0564, USA.
SOURCE: TRENDS IN PHARMACOLOGICAL SCIENCES, (2001 Sep) 22 (9) 471-7. Ref: 44
Journal code: 7906158. ISSN: 0165-6147.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, MULTICASE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200110
ENTRY DATE: Entered STN: 20010910
Last Updated on STN: 20011029
Entered Medline: 20011025

AB For each alpha(2)-adrenoceptor subtype (alpha(2A), alpha(2B) and alpha(2C)), sequence variations within the coding region of each gene have been identified in humans. These result in substitutions or **deletions** of amino acids in the third **intracellular loops** of each receptor. This article summarizes the genetics and molecular biology of alpha(2)-adrenoceptor polymorphisms, including the consequences of each polymorphism on receptor signaling, as determined in transfected cells. These effects include alterations in **G-protein** coupling, **desensitization** and **G-protein** receptor kinase-mediated phosphorylation. Studies so far provide the mechanistic basis for future studies to investigate genetic risk factors and pharmacogenetics in pathophysiological conditions linked to alpha(2)-adrenoceptor function.

L10 ANSWER 3 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:491249 BIOSIS
DOCUMENT NUMBER: PREV200100491249
TITLE: A novel **yeast**-based assay facilitates direct mapping of the receptor-**G protein** interface: Application to serotonin receptors.
AUTHOR(S): Shapiro, D. A. (1); Kroeze, W. K. (1); Weiner, D. M.; Roth, B. L. (1)
CORPORATE SOURCE: (1) Biochemistry, Case Western Reserve Univ, Cleveland, OH USA
SOURCE: Society for Neuroscience Abstracts, (2001) Vol. 27, No. 1, pp. 694. print.
Meeting Info.: 31st Annual Meeting of the Society for Neuroscience San Diego, California, USA November 10-15, 2001
ISSN: 0190-5295.
DOCUMENT TYPE: Conference
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The molecular and atomic mechanisms by which receptors interact with G proteins are unknown, but are being intensely studied. We have devised a novel **yeast**-based method to directly determine the residues involved in 5-HT2A receptor-Gq interactions. Using this **yeast** -based assay system we tested a model based on prior mutagenesis studies which suggested that a series of residues (316-328) at the extreme C-terminus of the third **intracellular loop** (i3) of the 5-HT2A receptor are involved in 5-HT2A-Gq interactions. The **yeast** -based assay system has directly verified that the extreme C-terminus of the i3 loop can physically interact with Gq. Based on these results, and ongoing model-building efforts, predictions were made concerning the relative constitutive activity of a series of i3 C-terminus mutants. We were able to confirm the predictions derived from the **yeast** -based assay system using the mammalian cell based functional assay R-SAT (Receptor-Stimulated Activation of Transcription). The combined results from the mammalian and **yeast**-based assays allow us to now predict residues which modulate the constitutive activity of S-HT2A receptors via either direct or indirect (i.e. allosteric) mechanisms. It is likely that this approach will be generally applicable to studies of receptor-**G protein** interactions.

L14 ANSWER 11 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1998:473975 BIOSIS
DOCUMENT NUMBER: PREV199800473975
TITLE: **Heterologous** expression of **G-protein**-coupled receptors: Human opioid receptors under scrutiny.
AUTHOR(S): Stanasila, L.; Pattus, F.; Massotte, D. (1)
CORPORATE SOURCE: (1) Departement des Recepteurs et Proteines Membranaires, UPR 9050 CNRS, Ecole Superieure de Biotechnologie de Strasbourg, 67400 Illkirch-Graffenstaden France
SOURCE: Biochimie (Paris), (**May-June, 1998**) Vol. 80, No. 5-6, pp. 563-571.
ISSN: 0300-9084.
DOCUMENT TYPE: General Review
LANGUAGE: English
AB **G-protein**-coupled receptors whose topology shows **seven transmembrane** domains form the largest known family of receptors involved in higher organism signal transduction. Despite increasing knowledge on the functioning mechanisms of these receptors, almost no structural data are available but only a few models. Structural studies using a wide range of physical and biochemical techniques may require fairly large (up to several milligrams) amounts of purified protein. Since such quantities are not naturally available, overexpression is prerequisite. **Heterologous** expression systems are then assayed for maximal production of a protein facsimile. **Heterologous** systems may also provide interesting alternatives for receptor functional studies in a different cellular context. Opioid receptors will be used as an example to discuss aspects related to the choice and suitability of several different expression systems for the intended analysis of **G-protein**-coupled receptor properties. General implications will be outlined.

L14 ANSWER 9 OF 14 MEDLINE

ACCESSION NUMBER: 89214226 MEDLINE
DOCUMENT NUMBER: 89214226 PubMed ID: 2540202
TITLE: Increasing gene expression in **yeast** by fusion to ubiquitin.
AUTHOR: Ecker D J; Stadel J M; Butt T R; Marsh J A; Monia B P; Powers D A; Gorman J A; Clark P E; Warren F; Shatzman A; +
CORPORATE SOURCE: Department of Molecular Pharmacology, Smith Kline & French Laboratories, King of Prussia, Pennsylvania 19406-0939.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1989 May 5)** 264 (13) 7715-9.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198906
ENTRY DATE: Entered STN: 19900306
Last Updated on STN: 20000303
Entered Medline: 19890602

AB **Heterologous** gene expression in **yeast** can be increased up to several hundred-fold by expressing a foreign gene as a fusion to the ubiquitin gene. An endogenous **yeast** endoprotease (Ub-Xase) removes the ubiquitin from the fusion product to produce the authentic protein. The utility of this technique has been demonstrated by expression of three different proteins in **yeast** as both unfused and ubiquitin-fused forms: 1) the alpha subunit of the mammalian stimulating **G-protein** of the adenylate cyclase complex (Gs alpha); 2) a soluble fragment of the T cell receptor protein (sCD4); and 3) the protease domain of human urokinase (UKP). The sequence specificity of the Ub-Xase was demonstrated by mutagenesis of the carboxyl-terminal glycine of ubiquitin to an alanine, which inhibited ubiquitin removal in vivo. Processing of the ubiquitin-Gs alpha fusion protein (ub-Gs alpha) in vivo resulted in Gs alpha which could be reconstituted in mammalian membrane preparations and had the same specific activity as the authentic Gs alpha expressed in **yeast**. The **yeast** Ub-Xase has also been shown to work in vitro by the processing of a ub-sCD4 fusion protein synthesized in Escherichia coli. This technology should greatly enhance the utility of **yeast** for **heterologous** protein production.

L14 ANSWER 7 OF 14 MEDLINE
ACCESSION NUMBER: 90306353 MEDLINE
DOCUMENT NUMBER: 90306353 PubMed ID: 2194839
TITLE: Expression and pharmacological characterization of the human M1 muscarinic receptor in Saccharomyces cerevisiae.
AUTHOR: Payette P; Gossard F; Whiteway M; Dennis M
CORPORATE SOURCE: National Research Council of Canada, Biotechnology Research Institute, Montreal, Que.
SOURCE: FEBS LETTERS, **(1990 Jun 18)** 266 (1-2) 21-5.
Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199008
ENTRY DATE: Entered STN: 19900921
Last Updated on STN: 19980206
Entered Medline: 19900814

AB The **yeast** S. cerevisiae has been examined as a **heterologous** host for the expression of mammalian neurotransmitter receptors which couple to guanine nucleotide regulatory (G) proteins. A cloned gene encoding the M1 subtype of human muscarinic receptor (HM1) was transformed into S. cerevisiae on a high copy plasmid under the control of the promoter for the **yeast** alcohol dehydrogenase (ADH) gene. Northern blotting demonstrated the presence of HM1 transcripts in transformants, and crude membranes prepared from these cells showed saturable binding of the muscarinic antagonist [3H]N-methyl scopolamine with a Kd of 179 pM and Bmax of 20 fmol/mg protein. Competition binding studies revealed pharmacological properties for these sites which were comparable to those reported for the M1 site in mammalian tissues. **Yeast** expressing HM1 did not exhibit high affinity agonist binding or cell-cycle arrest in the presence of muscarinic agonists, indicating that the mammalian receptor did not couple to the endogenous **yeast G protein**.

L14 ANSWER 6 OF 14 MEDLINE
ACCESSION NUMBER: 96021603 MEDLINE
DOCUMENT NUMBER: 96021603 PubMed ID: 8533467
TITLE: Targeting of **heterologous** membrane proteins into proliferated internal membranes in Saccharomyces cerevisiae.
AUTHOR: Wittekindt N E; Wurgler F E; Sengstag C
CORPORATE SOURCE: Institute of Toxicology, Swiss Federal Institute of Technology, Schwerzenbach, Switzerland.
SOURCE: YEAST, **(1995 Aug)** 11 (10) 913-28.
Journal code: 8607637. ISSN: 0749-503X.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199601
ENTRY DATE: Entered STN: 19960220
Last Updated on STN: 19960220
Entered Medline: 19960126

AB Overproduction of chimeric proteins containing the HMG2/1 peptide, which comprises the **seven transmembrane** domains of Saccharomyces cerevisiae 3-hydroxy-3-methylglutaryl-CoA reductase isozymes 1 and 2, has previously been observed to induce the proliferation of internal endoplasmic reticulum-like membranes. In order to exploit this amplified membrane surface area for the accommodation of **heterologous** microsomal proteins, we fused sequences coding for human cytochrome P4501A1 (CYP1A1) to sequences encoding the HMG2/1 peptide and expressed the hybrid genes in **yeast**. The **heterologous** hybrid proteins were targeted into strongly proliferated membranes, as shown by electron microscopic and immunofluorescent analysis. Fusion proteins comprising the whole CYP1A1 polypeptide (HMG2/1-CYP1A1) exhibited 7-ethoxyresorufin-O-deethylase activity, whereas fusion proteins lacking the N-terminal 56 amino acids of CYP1A1 (HMG2/1-delta CYP1A1) were inactive and appeared to be unable to incorporate protoheme. Similar amounts of **heterologous** protein were detected in cells expressing HMG2/1-CYP1A1, HMG2/1-delta CYP1A1 and CYP1A1, respectively. Replacement of the N-terminal membrane anchor domain of human NADPH-cytochrome P450 oxidoreductase by the HMG2/1 peptide also resulted in a functional fusion enzyme, which was able to interact with HMG2/1-CYP1A1 and the **yeast** endogenous P450 enzyme lanosterol-14 alpha-demethylase.

L14 ANSWER 5 OF 14 MEDLINE
ACCESSION NUMBER: 97400498 MEDLINE
DOCUMENT NUMBER: 97400498 PubMed ID: 9252333
TITLE: Gpa2p, a **G-protein** alpha-subunit, regulates growth and pseudohyphal development in Saccharomyces cerevisiae via a cAMP-dependent mechanism.
AUTHOR: Kubler E; Mosch H U; Rupp S; Lisanti M P
CORPORATE SOURCE: Institut fur Mikrobiologie, Georg-August-Universitat Gottingen, D-37077 Gottingen, Germany.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1997 Aug 15)** 272 (33) 20321-3.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 19970916
Last Updated on STN: 20000303
Entered Medline: 19970904

AB The small GTP-binding protein Ras and heterotrimeric G-proteins are key regulators of growth and development in eukaryotic cells. In mammalian cells, Ras functions to regulate the mitogen-activated protein kinase pathway in response to growth factors, whereas many heterotrimeric GTP-binding protein alpha-subunits modulate cAMP levels through adenylyl cyclase as a consequence of hormonal action. In contrast, in the **yeast** Saccharomyces cerevisiae, it is the Ras1 and Ras2 proteins that regulate adenylyl cyclase. Of the two **yeast G-protein** alpha-subunits (GPA1 and GPA2), only GPA1 has been well studied and shown to negatively regulate the mitogen-activated protein kinase pathway upon pheromone stimulation. In this report, we show that deletion of the GPA2 gene encoding the other **yeast G-protein** alpha-subunit leads to a defect in pseudohyphal development. Also, the GPA2 gene is indispensable for normal growth in the absence of Ras2p. Both of these phenotypes can be rescued by deletion of the PDE2 gene product, which inactivates cAMP by cleavage, suggesting that these phenotypes can be attributed to low levels of intracellular cAMP. In support of this notion, addition of exogenous cAMP to the growth media was also sufficient to rescue the phenotype of a GPA2 deletion strain. Taken together, our results directly demonstrate that a **G-protein** alpha-subunit can regulate the growth and pseudohyphal development of S. cerevisiae via a cAMP-dependent mechanism. **Heterologous** expression of mammalian **G-protein** alpha-subunits in these **yeast** GPA2 deletion strains could provide a valuable tool for the mutational analysis of mammalian **G-protein** function in an in vivo null setting.

L14 ANSWER 2 OF 14 MEDLINE
ACCESSION NUMBER: 1998438568 MEDLINE
DOCUMENT NUMBER: 98438568 PubMed ID: 9763517
TITLE: N-terminal domain of Gpa1 (**G protein** alpha) subunit) is sufficient for plasma membrane targeting in **yeast** Saccharomyces cerevisiae.
AUTHOR: Gillen K M; Pausch M; Dohlman H G
CORPORATE SOURCE: Department of Pharmacology, Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, Connecticut 06536-0812, USA.
CONTRACT NUMBER: GM 55316 (NIGMS)
T 32CA 09085 (NCI)
SOURCE: JOURNAL OF CELL SCIENCE, **(1998 Nov)** 111 (Pt 21) 3235-44.
Journal code: 0052457. ISSN: 0021-9533.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990324
Last Updated on STN: 20000303
Entered Medline: 19990309

AB G proteins play a central role in transmitting signals from cell surface receptors to effector proteins inside the cell. Signaling can only occur, however, if all these protein components are properly assembled and localized at the plasma membrane. Past studies have shown that certain segments within the N-terminal region of the **G protein** alpha subunit are necessary for membrane attachment. Here we identify a region within the **yeast** G alpha (Gpa1) that is sufficient for membrane attachment, as well as for specific targeting to the plasma membrane. Initially, we constructed chimeric proteins that replace the N terminus of mammalian Gsalpha with the corresponding sequence from Gpa1. Gsalpha is inefficiently targeted to the **yeast** plasma membrane and therefore cannot fully complement the loss of Gpa1. Gpa1-Gsaplha chimeras were assayed for proper membrane localization by functional complementation of a gpa1Delta ;) mutant, and by sucrose density gradient fractionation of cell membranes. Most of the chimeras tested, including one with only the N-terminal 7 amino acids from Gpa1, exhibited normal membrane targeting and complementing activity. We also fused various lengths of N-terminal Gpa1 sequence to glutathione-S-transferase (GST), a **heterologous** protein normally expressed in the cytoplasm. The first 67- 36- or 9-amino acids of Gpa1 were all sufficient to direct GST specifically to the plasma membrane in **yeast**. This analysis defines the extreme N terminus of Gpa1 as the primary determinant of proper membrane targeting, and represents an essential step towards isolating and identifying **G protein**-targeting proteins within the plasma membrane.

L14 ANSWER 1 OF 14 MEDLINE
ACCESSION NUMBER: 1999038722 MEDLINE
DOCUMENT NUMBER: 99038722 PubMed ID: 9821281
TITLE: Production of **G-protein**-coupled receptors in **yeast**.
AUTHOR: Reilander H; Weiss H M
CORPORATE SOURCE: Max-Planck-Institut fur Biophysik, Abteilung Molekulare Membranbiologie, Frankfurt/M., Germany.. reilaender@mpibp-frankfurt.mpg.de
SOURCE: CURRENT OPINION IN BIOTECHNOLOGY, **(1998 Oct)** 9 (5) 510-7. Ref: 46
Journal code: 9100492. ISSN: 0958-1669.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 20000303
Entered Medline: 19981211

AB Yeasts combine the advantages of fast and easy handling with the potential to perform eukaryotic post-translational modifications and are for this reason interesting hosts for **heterologous** production of **G-protein**-coupled receptors. The possibility to connect foreign receptors to a **yeast** internal MAP kinase pathway was used to establish **yeast**-based systems for high-throughput screening of compound libraries. In addition, yeasts have the potential for high level production of **G-protein**-coupled receptors. In this field, non-Saccharomyces yeasts seems to be interesting alternatives to S. cerevisiae, as well as to systems based on higher eukaryotic cells.

L18 ANSWER 11 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1995:73196 BIOSIS
DOCUMENT NUMBER: PREV199598087496
TITLE: Activation of a GTP-binding protein and a GTP-binding-protein-coupled receptor kinase (beta-adrenergic-receptor kinase-1) by a **muscarinic** receptor m2 mutant lacking phosphorylation sites.
AUTHOR(S): Kameyama, Kimihiko (1); Haga, Kazuko; Haga, Tatsuya; Moro, Osamu; Sadee, Wolfgang
CORPORATE SOURCE: (1) Dep. Biochem., Inst. Brain Res., Fac. Med., Univ. Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113 Japan
SOURCE: European Journal of Biochemistry, (1994) Vol. 226, No. 2, pp. 267-276.
ISSN: 0014-2956.
DOCUMENT TYPE: Article
LANGUAGE: English
AB A mutant of the human **muscarinic** acetylcholine receptor m2 subtype (m2 receptor), lacking a large part of the third **intracellular loop**, was expressed and purified using the baculovirus/insect cell culture system. The mutant was not phosphorylated by beta-adrenergic-receptor kinase, as expected from the previous assignment of phosphorylation sites to the central part of the third **intracellular loop**. However, the m2 receptor mutant was capable of stimulating beta-adrenergic-receptor-kinase-1-mediated phosphorylation of a glutathione S-transferase fusion protein containing the m2 phosphorylation sites in an agonist-dependent manner. Both mutant and wild-type m2 receptors reconstituted with the guanine-nucleotide-binding regulatory proteins (G protein), G-o and G-i2, displayed guanine-nucleotide-sensitive high-affinity agonist binding, as assessed by displacement of (3H)quinuclidinylbenzilate binding with carbamoylcholine, and both stimulated guanosine 5'-3-O-(35S)thiotriphosphate ((35S)GTP(S)) binding in the presence of carbamoylcholine and GDP. The K-i values of carbamoylcholine effects on (3H)quinuclidinyl-benzilate binding were indistinguishable for the mutant and wild-type m2 receptors. Moreover, the phosphorylation of the wild-type m2 receptor by beta-adrenergic-receptor kinase-1 did not affect m2 interaction with G proteins as assessed by the binding of (3H)quinuclidinyl benzilate or (35S)GTP(S). These results indicate that (a) the m2 receptor serves both as an activator and as a substrate of beta-adrenergic-receptor kinase, and (b) a large part of the third **intracellular loop** of the m2 receptor does not contribute to interaction with G proteins and its phosphorylation by beta-adrenergic-receptor kinase does not uncouple the receptor and G proteins in reconstituted lipid vesicles.

L18 ANSWER 10 OF 12 MEDLINE
ACCESSION NUMBER: 92317060 MEDLINE
DOCUMENT NUMBER: 92317060 PubMed ID: 1618842
TITLE: Hm1 **muscarinic** cholinergic receptor internalization requires a domain in the third cytoplasmic loop.
AUTHOR: Lameh J; Philip M; Sharma Y K; Moro O; Ramachandran J; Sadee W
CORPORATE SOURCE: Department of Pharmacy, University of California, San Francisco 94143.
CONTRACT NUMBER: DA 04166 (NIDA)
GM 43102 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1992 Jul 5)** 267 (19) 13406-12.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199208
ENTRY DATE: Entered STN: 19920815
Last Updated on STN: 19980206
Entered Medline: 19920805

AB Selected regions of the Hm1 **muscarinic** cholinergic receptor were mutated to analyze the molecular mechanisms of agonist-induced receptor internalization (or sequestration). The wild-type and mutant Hm1 genes were expressed, using pSG5, in U293 human kidney cells. Whereas surface receptor density measured with the polar tracer N-[3H]methylscopolamine was rapidly reduced by carbachol exposure, total receptor content measured with [3H]quinuclidinyl benzilate did not decline for at least 24 h, indicating the absence of extensive receptor down-regulation in U293 cells. Carbachol stimulation of phosphatidylinositol turnover paralleled receptor internalization, both with EC50 values of 10-20 microM. Furthermore, a D71N point mutation that prevented receptor activation also abolished carbachol-induced receptor internalization, indicating that receptor activation (but not necessarily second messenger stimulation) was required for internalization. Truncation of the COOH-terminal tail (K447 trunc) and point mutations of several potential Ser and Thr phosphorylation sites to Ala failed to affect receptor activation and internalization. In contrast, partial **deletions** of the third **intracellular loop** (i3) (Tyr208-Thr366) resulted in receptor mutants deficient in agonist-induced receptor internalization/sequestration. Various **deletions** caused either complete loss of internalization (d 232-358) or impaired internalization, ranging from 10 to 30% over 2 h, whereas wild-type Hm1 internalized to approximately 50%. Whereas the reason for the observed differences among the deficient **deletion** mutants remains unclear, the initial rate of N-[3H]methylscopolamine binding loss from the cell surface was much slower than that of wild-type Hm1 in each case. The **deletion** of only one single domain, 284-292 (SMESLTSSE), in the middle of i3 was consistently associated with impaired internalization. Domain 284-292 is partially conserved among closely related **muscarinic** receptors, whereas most of the remainder of i3 is not (except for the i3 membrane junctions), and similar Ser- and Thr-rich regions are present in many other G protein-coupled receptors. We propose that a small receptor domain in the middle of the i3 loop of Hm1 is involved in agonist-induced receptor internalization.

L18 ANSWER 5 OF 12 MEDLINE
ACCESSION NUMBER: 96081975 MEDLINE
DOCUMENT NUMBER: 96081975 PubMed ID: 7499433
TITLE: Desensitization and internalization of the m2 **muscarinic** acetylcholine receptor are directed by independent mechanisms.
AUTHOR: Pals-Rylaarsdam R; Xu Y; Witt-Enderby P; Benovic J L; Hosey M M
CORPORATE SOURCE: Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, Illinois 60611, USA.
CONTRACT NUMBER: ES00210 (NIEHS)
GM 44944 (NIGMS)
HL 50201 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1995 Dec 1)** 270 (48) 29004-11.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199601
ENTRY DATE: Entered STN: 19960217
Last Updated on STN: 19960217
Entered Medline: 19960118

AB The phenomenon of acute desensitization of G-protein-coupled receptors has been associated with several events, including receptor phosphorylation, loss of high affinity agonist binding, receptor:G-protein uncoupling, and receptor internalization. However, the biochemical events underlying these processes are not fully understood, and their contributions to the loss of signaling remain correlative. In addition, the nature of the kinases and the receptor domains which are involved in modulation of activity have only begun to be investigated. In order to directly measure the role of G-protein-coupled receptor kinases (GRKs) in the desensitization of the m2 **muscarinic** acetylcholine receptor (m2 mAChR), a dominant-negative allele of GRK2 was used to inhibit receptor phosphorylation by endogenous GRK activity in a human embryonic kidney cell line. The dominant-negative GRK2K220R reduced agonist-dependent phosphorylation of the m2 mAChR by approximately 50% and prevented acute desensitization of the receptor as measured by the ability of the m2 mAChR to attenuate adenylyl cyclase activity. In contrast, the agonist-induced internalization of the m2 mAChR was unaffected by the GRK2K220R construct. Further evidence linking receptor phosphorylation to acute receptor desensitization was obtained when two **deletions** of the third **intracellular loop** were made which created m2 mAChRs that did not become phosphorylated in an agonist-dependent manner and did not desensitize. However, the mutant mAChRs retained the ability to internalize. These data provide the first direct evidence that GRK-mediated receptor phosphorylation is necessary for m2 mAChR desensitization; the likely sites of in vivo phosphorylation are in the central portion of the third **intracellular loop** (amino acids 282-323). These results also indicate that internalization of the m2 receptor is not a key event in desensitization and is mediated by mechanisms distinct from GRK phosphorylation of the receptor.

L18 ANSWER 4 OF 12 MEDLINE
ACCESSION NUMBER: 96198073 MEDLINE
DOCUMENT NUMBER: 96198073 PubMed ID: 8626406
TITLE: Molecular mechanisms involved in **muscarinic** acetylcholine receptor-mediated G protein activation studied by insertion mutagenesis.
AUTHOR: Liu J; Blin N; Conklin B R; Wess J
CORPORATE SOURCE: Laboratory of Bioorganic Chemistry, NIDDK, National Institutes of Health, Bethesda, Maryland 20892, USA.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1996 Mar 15)** 271 (11) 6172-8.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199606
ENTRY DATE: Entered STN: 19960708
Last Updated on STN: 20000303
Entered Medline: 19960624

AB We have recently shown that a four-amino acid epitope (VTIL) on the m2 **muscarinic** receptor (corresponding to Val385, Thr386, Ile389, and Leu390) is essential for Gi/o coupling specificity and Gi/o activation (Liu, J., Conklin, B. R., Blin, N., Yun, J., and Wess, J. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 11642-11646). Because this sequence element is thought to be located at the junction between the third **intracellular loop** and the sixth transmembrane helix (TM VI), we speculated that agonist binding to the m2 receptor protein results in conformational changes that enable the VTIL motif to interact with Gi/o proteins. To test the hypothesis that such structural changes might involve a relative movement of TM VI toward the cytoplasm, we created a series of mutant m2 **muscarinic** receptors in which one to four extra Ala residues were inserted into TM VI immediately after Leu390. Based on the geometry of an alpha-helix, such mutations are predicted to "push" the VTIL sequence away from the lipid bilayer. Consistent with our working hypothesis, second messenger assays with transfected COS-7 cells showed that all mutant m2 receptors containing extra Ala residues C-terminal of Leu390 could activate the proper G proteins even in the absence of agonist. However, replacement of the VTIL motif in such constitutively active m2 receptors with the corresponding m3 **muscarinic** receptor sequence (AALS) or **deletion** of Ala391 from the wild type m2 receptor completely abolished G protein coupling. Interestingly, introduction of extra Ala residues C-terminal of the AALS motif in the m3 **muscarinic** receptor completely abolished functional activity. Mutant m2 and m3 receptors that contained extra Ala residues immediately N-terminal of the VTIL and AALS motif, respectively, displayed wild type-like coupling properties. Our data are consistent with a model in which agonist binding to the m2 **muscarinic** receptor leads to a relative movement of TM VI toward the cytoplasm, thus enabling the adjacent VTIL sequence to interact with the C terminus of Galpha(i/o) subunits.

L18 ANSWER 2 OF 12 MEDLINE
ACCESSION NUMBER: 97347289 MEDLINE
DOCUMENT NUMBER: 97347289 PubMed ID: 9203628
TITLE: The role of the aspartate-arginine-tyrosine triad in the m1 **muscarinic** receptor: mutations of aspartate 122 and tyrosine 124 decrease receptor expression but do not abolish signaling.
AUTHOR: Lu Z L; Curtis C A; Jones P G; Pavia J; Hulme E C
CORPORATE SOURCE: Division of Physical Biochemistry, Medical Research Council National Institute for Medical Research, London, UK.
SOURCE: MOLECULAR PHARMACOLOGY, **(1997 Feb)** 51 (2) 234-41.
Journal code: 0035623. ISSN: 0026-895X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199707
ENTRY DATE: Entered STN: 19970805
Last Updated on STN: 19970805
Entered Medline: 19970723

AB An Asp-Arg-Tyr triad occurs in a majority of rhodopsin-like G protein-coupled receptors. The fully conserved Arg is critical for G protein activation, but the function of the flanking residues is not well understood. We expressed in COS-7 cells m1 **muscarinic** receptors that were mutated at Asp122 and Tyr124. Most mutations at either position strongly attenuated or prevented the expression of binding sites for the antagonist [3H]N-methylscopolamine. However, sites that were expressed displayed unaltered affinity for the antagonist. Receptor protein, visualized with a carboxyl-terminally directed antibody, was reduced but never completely abolished. The effects of these mutations were partially reversed by the **deletion** of 129 amino acids from the third **intracellular loop** of the receptor. In several cases, comparison of immunocytochemistry with binding measurements suggested the presence of substantial amounts of inactive, presumably misfolded, receptor protein. Some of the variants that bound [3H]N-methylscopolamine underwent small changes in their affinities for acetylcholine. All retained nearly normal abilities to mediate an acetylcholine-induced phosphoinositide response. We propose that Asp122 and Tyr124 make intramolecular contacts whose integrity is important for efficient receptor folding but that they do not participate directly in signaling. The role of these residues is completely distinct from that of Arg123, whose mutation abolishes signaling without diminishing receptor expression.

L21 ANSWER 15 OF 17 MEDLINE
ACCESSION NUMBER: 90306353 MEDLINE
DOCUMENT NUMBER: 90306353 PubMed ID: 2194839
TITLE: Expression and pharmacological characterization of the human M1 **muscarinic** receptor in Saccharomyces cerevisiae.
AUTHOR: Payette P; Gossard F; Whiteway M; Dennis M
CORPORATE SOURCE: National Research Council of Canada, Biotechnology Research Institute, Montreal, Que.
SOURCE: FEBS LETTERS, **(1990 Jun 18)** 266 (1-2) 21-5.
Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199008
ENTRY DATE: Entered STN: 19900921
Last Updated on STN: 19980206
Entered Medline: 19900814

AB The **yeast** S. cerevisiae has been examined as a heterologous host for the expression of mammalian neurotransmitter receptors which couple to guanine nucleotide regulatory (G) proteins. A cloned gene encoding the M1 subtype of human **muscarinic** receptor (HM1) was transformed into S. cerevisiae on a high copy plasmid under the control of the promoter for the **yeast** alcohol dehydrogenase (ADH) gene. Northern blotting demonstrated the presence of HM1 transcripts in transformants, and crude membranes prepared from these cells showed saturable binding of the **muscarinic** antagonist [3H]N-methyl scopolamine with a Kd of 179 pM and Bmax of 20 fmol/mg protein. Competition binding studies revealed pharmacological properties for these sites which were comparable to those reported for the M1 site in mammalian tissues. **Yeast** expressing HM1 did not exhibit high affinity agonist binding or cell-cycle arrest in the presence of **muscarinic** agonists, indicating that the mammalian receptor did not couple to the endogenous **yeast** G protein.

L21 ANSWER 13 OF 17 MEDLINE
ACCESSION NUMBER: 92171926 MEDLINE
DOCUMENT NUMBER: 92171926 PubMed ID: 1540163
TITLE: Functional expression of rat M5 **muscarinic** acetylcholine receptor in **yeast**.
AUTHOR: Huang H J; Liao C F; Yang B C; Kuo T T
CORPORATE SOURCE: Graduate Institute of Botany, National Taiwan University, Taipei, Republic of China.
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, **(1992 Feb 14)** 182 (3) 1180-6.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199203
ENTRY DATE: Entered STN: 19920417
Last Updated on STN: 19980206
Entered Medline: 19920327

AB We have produced the rat M5 **muscarinic** acetylcholine receptor, an integral membrane protein, in the **yeast** Saccharomyces cerevisiae. This was achieved by placing an M5 gene in the **yeast** vector under the control of the **yeast** alpha-factor promoter and leader sequence. Northern blotting revealed the presence of M5 transcripts in **yeast** transformed with the M5 plasmid constructs. Crude extract prepared from the transformant yeasts showed saturable binding of the **muscarinic** antagonist [3H]-N-methyl scopolamine ([3H]NMS) with a kd of 22.77 nM and Bmax of 134.76 fmole per mg protein. Results deduced from saturation binding assay of intact cell demonstrated clearly that the M5 receptor was translocated across the membrane of the endoplasmic reticulum using the secretion signal on alpha-leader sequence and its binding site was still functional. **Yeast** expressing M5 receptor did not exhibit cell-cycle arrest in the presence of carbachol, a acetylcholine agonist, indicating that the recombinant M5 receptor could not couple directly to the endogenous **yeast** pheromone signaling G-protein.

L21 ANSWER 11 OF 17 MEDLINE
ACCESSION NUMBER: 92394963 MEDLINE
DOCUMENT NUMBER: 92394963 PubMed ID: 1522111
TITLE: Pathways of internalization of the hCG/LH receptor: immunoelectron microscopic studies in Leydig cells and transfected L-cells.
AUTHOR: Ghinea N; Vu Hai M T; Groyer-Picard M T; Houllier A; Schoevaert D; Milgrom E
CORPORATE SOURCE: Institut National de la Sante et de la Recherche Medicale, Unite 135, Le Kremlin-Bicetre, France.
SOURCE: JOURNAL OF CELL BIOLOGY, **(1992 Sep)** 118 (6) 1347-58.
Journal code: 0375356. ISSN: 0021-9525.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199210
ENTRY DATE: Entered STN: 19921023
Last Updated on STN: 19980206
Entered Medline: 19921014

AB Monoclonal anti-receptor antibodies were used to study the cellular traffic of the hCG/LH receptor by immunoelectron microscopy. The LHR38 antibody was shown to bind to the extracellular domain of the receptor but not to interfere with hormone binding, adenylate cyclase activation or with the rate of internalization of the receptor. Pig Leydig cells and a permanent L-cell line expressing the LH receptor were used for the study. Incubation with LHR38-gold complexes showed the LH receptors to be randomly distributed over the cell surface including the clathrin coated pits. The LH receptors were internalized via a route including coated pits, coated vesicles and multivesicular bodies to lysosomes. This route is different from that observed for beta-adrenergic, **muscarinic**, and **yeast** mating factor receptors and considered previously as possibly general for G-protein-coupled receptors. The use of [125I]LHR38 allowed precise measurement of the rate of internalization, showing the existence of a constitutive pathway which was increased 11-fold by hormone administration. Double labeling experiments suggested that the hormone (hCG-Au15nm) and the receptor (labeled with LHR38-Au5nm) have similar routes of endocytosis, both of them being degraded in lysosomes. Studies of the reappearance of LHR38-Au5nm on the surface of the cells and the use of monensin indicated that only a very small proportion of the receptor molecules were recycled to the cell surface. The distribution and the intracellular pathways of LH receptors are very similar in Leydig cells and transfected L-cells. This opens the possibility of using the latter to study, by in vitro mutagenesis, the molecular mechanisms involved in the cellular traffic of LH receptors.

L21 ANSWER 10 OF 17 MEDLINE
ACCESSION NUMBER: 94240188 MEDLINE
DOCUMENT NUMBER: 94240188 PubMed ID: 8183960
TITLE: **Yeast** as a model system for mammalian seven-transmembrane segment receptors.
AUTHOR: Jeansonne N E
CORPORATE SOURCE: East Carolina University Medical School, Department of Pharmacology, Greenville, North Carolina 27858.
SOURCE: PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, **(1994 May)** 206 (1) 35-44. Ref: 115
Journal code: 7505892. ISSN: 0037-9727.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199406
ENTRY DATE: Entered STN: 19940621
Last Updated on STN: 19940621
Entered Medline: 19940614

AB Investigators have used the budding **yeast** Saccharomyces cerevisiae as a model system in which to study the beta-adrenergic receptor, the T-cell receptor pathway, initiation of mammalian DNA replication, initiation of mammalian transcription, secretion, the CDC2 kinase system, cell cycle control, and aging, as well as the function of oncogenes. This list continues to grow with the discovery of an immunoglobulin heavy-chain binding homologue in **yeast**, an Rb binding protein homologue, and a possible **yeast** arrestin. **Yeast** is relatively easy to maintain, to grow, and to genetically manipulate. A single gene can be overexpressed, selectively mutated or deleted from its chromosomal location. In this way, the in vivo function of a gene can be studied. It has become reasonable to consider **yeast** as a model system for studying the seven transmembrane segments (7-TMS) receptor family. Currently, subtypes of the beta-adrenergic receptor are being studied in **yeast**. The receptor and its G alpha-G-protein, trigger the mating pheromone receptor pathway. This provides a powerful assay for determining receptor function. Studies expressing the **muscarinic** cholinergic receptor in **yeast** are underway. The **yeast** pheromone receptor belongs to this receptor family, sharing sequence and secondary structure homology. An effective strategy has been to identify a **yeast** pathway or process which is homologous to a mammalian system. The pathway is delineated in **yeast**, identifying other genetic components. Then **yeast** genes are used to screen for human homologues of these components. The putative human homologues are then expressed in **yeast** and in mammalian cells to determine function. When this type of "mixing and matching" works, **yeast** genetics can be a powerful tool.

L21 ANSWER 7 OF 17 MEDLINE
ACCESSION NUMBER: 95075488 MEDLINE
DOCUMENT NUMBER: 95075488 PubMed ID: 7984268
TITLE: The molecular evolution of G protein-coupled receptors: focus on 5-hydroxytryptamine receptors.
AUTHOR: Peroutka S J; Howell T A
CORPORATE SOURCE: Palo Alto Institute for Molecular Medicine, Hillsborough, CA 94010.
CONTRACT NUMBER: NS 25360-07 (NINDS)
SOURCE: NEUROPHARMACOLOGY, **(1994 Mar-Apr)** 33 (3-4) 319-24.
Journal code: 0236217. ISSN: 0028-3908.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Space Life Sciences
ENTRY MONTH: 199412
ENTRY DATE: Entered STN: 19950116
Last Updated on STN: 20000303
Entered Medline: 19941230

AB Phylogenetic comparisons between homologous proteins can provide information on the rates of molecular evolution of the proteins. G protein-coupled receptors are a "superfamily" of proteins which exist in species ranging from **yeast** to man. Based on an analysis of the percentage of amino acid homology between various species, the rate of molecular evolution of G protein-coupled receptors can be estimated at approx 1% per 10 million years. Based on this assumption, the primordial 5-HT receptor must have evolved more than 700-800 million years ago since the 3 major classes of G protein-coupled 5-HT receptors (i.e. 5-HT1, 5-HT2 and 5-HT6 receptors) are less than 25% homologous. 5-HT5, 5-HT7, 5-HTsnail, 5-HTdro and 5-HT1A receptors differentiated approx 600-700 million years ago, the time period during which vertebrates diverged from invertebrates. The mammalian 5-HT receptor subtypes have differentiated over the past 90 million years. Thus, although a recent flurry of "new" 5-HT receptors have appeared in the literature, the first "primordial" 5-HT receptor evolved over 750 million years ago, a date which likely predates the evolution of **muscarinic**, dopaminergic and adrenergic receptor systems. This analysis also predicts that a significant number of both mammalian and invertebrate G protein-coupled 5-HT receptor subtypes remain to be identified.

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=> d his

     (FILE 'HOME' ENTERED AT 12:16:34 ON 13 JUN 2003)

     FILE 'MEDLINE, BIOSIS' ENTERED AT 12:16:45 ON 13 JUN 2003
L1         57588 S (G-PROTEIN) OR GPCR OR (SEVEN TRANSMEMBR?)
L2           817 S L1 AND INTRACELLULAR LOOP?
L3           127 S L2 AND DELET?
L4            10 S L3 AND YEAST
L5             5 DUP REM L4 (5 DUPLICATES REMOVED)
L6            38 S L3 AND (INTERNALIZAT? OR DESENSITIZ?)
L7            21 DUP REM L6 (17 DUPLICATES REMOVED)
L8            27 S L2 AND YEAST
L9            17 S L8 NOT L4
L10           11 DUP REM L9 (6 DUPLICATES REMOVED)
L11         1277 S L1 AND YEAST
L12           52 S L11 AND HETEROLOGOUS
L13           36 DUP REM L12 (16 DUPLICATES REMOVED)
L14           14 S L13 AND PY<1999
L15          163 S MUSCARINIC AND (INTRACELLULAR LOOP OR IC3)
L16           33 S L15 AND (DELET? OR SUBST)
L17           19 DUP REM L16 (14 DUPLICATES REMOVED)
L18           12 S L17 AND PY<1999
L19           55 S MUSCARINIC AND YEAST
L20           33 DUP REM L19 (22 DUPLICATES REMOVED)
L21           17 S L20 AND PY<1999

=> log y
COST IN U.S. DOLLARS                            SINCE FILE      TOTAL
                                                   ENTRY     SESSION
FULL ESTIMATED COST                                120.97     121.18

STN INTERNATIONAL LOGOFF AT 13:31:06 ON 13 JUN 2003
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L7 ANSWER 1 OF 9 MEDLINE
ACCESSION NUMBER: 1998406068 MEDLINE
DOCUMENT NUMBER: 98406068 PubMed ID: 9733718
TITLE: The second intracellular loop of the m5 **muscarinic** receptor is the switch which enables G-protein coupling.
AUTHOR: Burstein E S; Spalding T A; Brann M R
CORPORATE SOURCE: ACADIA Pharmaceuticals Inc., San Diego, California 92121, USA.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1998 Sep 18)** 273 (38) 24322-7.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19981021
Last Updated on STN: 20000303
Entered Medline: 19981015

AB We have completed a systematic search of the intracellular loops of a **muscarinic** acetylcholine receptor for domains that govern G-protein coupling. A unique feature of the second intracellular (i2) loop was an ordered cluster of residues where diverse **substitutions** cause **constitutive** activation. A second group of residues in i2 was identified where mutations compromised receptor/G-protein coupling. The residues of each group alternate and are spaced three to four positions apart, suggesting an alpha-helical structure where these groups form opposing faces of the helix. We propose that the **constitutively** activating face normally constrains the receptor in the "off-state," while the other face couples G-proteins in the "on-state." Therefore, the i2 loop functions as the switch enabling G-protein activation.

L7 ANSWER 6 OF 9 MEDLINE
ACCESSION NUMBER: 96216352 MEDLINE
DOCUMENT NUMBER: 96216352 PubMed ID: 8621674
TITLE: Amino acid side chains that define **muscarinic** receptor/G-protein coupling. Studies of the third intracellular loop.
AUTHOR: Burstein E S; Spalding T A; Brann M R
CORPORATE SOURCE: Molecular Neuropharmacology Section, Department of Psychiatry, University of Vermont, Burlington VT 05405, USA.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1996 Feb 9)** 271 (6) 2882-5.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199606
ENTRY DATE: Entered STN: 19960627
Last Updated on STN: 20000303
Entered Medline: 19960619

AB Amino acids in the third intracellular loops of receptors play pivotal roles in G-protein coupling. To define their structural requirements, we have subjected the N- and C-terminal regions of this loop (Ni3 and Ci3, respectively) of the m5 **muscarinic** receptor to random saturation mutagenesis. (see Burstein, E. S., Spalding, T. A., Hill-Eubanks, D., and Brann, M. R. (1995) J. Biol. Chem. 270, 3141 3146 and Hill-Eubanks, D., Burstein, E. S., Spalding, T. A., Brauner-Osborne, H., and Brann, M. R. (1996) J. Biol. Chem. 271, 3058 3065). In the present study, we have extended our analysis of Ni3 by constructing libraries of receptors with all possible amino acid **substitutions** at the residues we previously identified as functionally important and characterizing their functional phenotypes. Numerous hydrophobic **substitutions** were well tolerated at Ile216 and Thr220 and caused **constitutive** activation in two cases, establishing that hydrophobicity is structurally favored at these positions and that many amino acid side chains are compatible with this structural role. Similarly, hydrophobic and polar, but not charged, **substitutions** were observed at Tyr217, but in contrast to results for Thr220, most **substitutions** at Tyr217 substantially decreased maximum response and increased the EC50 for carbachol, demonstrating that the specific side chain of residue 217 participates in G-protein coupling. Arg223 allowed the widest range of **substitutions** of the residues tested, but only basic residues were well tolerated. All other **substitutions** significantly increased (up to 100-fold) the EC50 for carbachol without significantly affecting maximal response. There were no significant changes in the ligand binding properties of these mutant receptors. We conclude that Ile216 and Thr220 fulfill a structural role, forming the foundation of the G-protein-coupling pocket, whereas Tyr217 and Arg223 contact G-proteins through specific side chain interactions. We propose that G-proteins are recruited to receptors by ionic interactions and that hydrophobic residues participate in activation.

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=> d his

     (FILE 'HOME' ENTERED AT 14:43:33 ON 13 JUN 2003)

     FILE 'MEDLINE, BIOSIS' ENTERED AT 14:43:45 ON 13 JUN 2003
L1         46354 S MUSCARINIC
L2             0 S L1 AND CONSTITUITIVE
L3         84083 S CONSTITUTIV? OR (AGONSIT INDEPENDENT)
L4           318 S L1 AND L3
L5            25 S L4 AND (SUBSTIT? OR DELET?)
L6            15 DUP REM L5 (10 DUPLICATES REMOVED)
L7             9 S L6 AND PY<1999

=>
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